A lifetime with Phenylketonuria

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Chapter 1

General Introduction

GENERAL INTRODUCTION

Phenylketonuria (PKU; OMIM 212600) is an inherited metabolic disorder. The deficient phenylalanine hydroxylase enzyme (PAH) prevents the conversion of phenylalanine (Phe) into tyrosine (Tyr). This causes an increase of blood and cerebral Phe and may lead to a cerebral shortage of other large neutral amino acids (LNAAs) such as Tyr and tryptophan. Tyr and tryptophan are metabolic precursors of the neurotransmitters dopamine and serotonin. Both dopamine and serotonin are related to several aspects of cognition and mental health. Untreated PKU has been associated with mental retardation, neurological problems (e.g. epilepsy), motor deficits and behavioural problems (Blau, van Spronsen, & Levy, 2010; DeRoche & Welsh, 2008; Smith & Knowles, 2000). As cognitive and behavioural problems are frequently observed in treated patients with PKU, monoamine shortages are generally considered to play an important role in the pathophysiology of PKU (Blau et al., 2010).

PKU is a rare genetic disorder affecting around 1 in every 10.000 newborn babies in Europe (Loeber, 2007), whereas this is approximately 1:15.000 in the USA (National Institutes of Health Consensus Development Panel, 2001). However, the prevalence varies largely between countries and regions due to differences in consanguinity (Blau et al., 2010). Neonatal screening is used for detecting PKU. In the Netherlands, neonatal screening for PKU was introduced in 1969 as a pilot in the Northern part of the Netherlands and nationwide in 1974 (Verkerk, 1995). Since then 10-15 newborns with PKU are diagnosed each year. Most patients are therefore treated early after birth, nowadays mostly within the first 10 days of life. Since 2002, Dutch PKU patients are treated according to national guidelines (van Spronsen, 2010). However, the key statements of the first European guidelines were recently developed and published (van Spronsen et al., 2017).

The severity of PAH deficiency in patients was generally based on the first measurement of blood Phe (i.e. pre-treatment Phe, at time of diagnosis). Healthy individuals have Phe concentrations between 50-110 μmol/L. PAH deficient patients were usually classified as follows (Blau et al., 2010; Blau, Hennermann, Langenbeck, & Lichter-Konecki, 2011): 'classic PKU' when untreated/pre-treatment Phe concentrations were above 1200 μmol/L; 'mild PKU' when untreated Phe concentrations were between 600-1200 μmol/L (a moderate classification was sometimes used with untreated Phe concentrations between 900-1200 μmol/L); 'non-PKU/mild hyperphenylalaninaemia' (HPA) with untreated Phe concentrations between 120-600 μmol/L. However, blood samples for neonatal screening may be collected before newborns achieve their peak Phe concentration. Additionally, there is discussion whether patients with untreated Phe concentrations between 120 and

360 μmol/L or those with untreated Phe concentrations of 120-600 μmol/L should be called mild HPA, suggesting that treatment in those patients is unnecessary. Therefore, the use of the traditional classification is deemed unsatisfactory and consequently, the European guidelines followed the proposal of Blau et al. (2011) towards a new classification system based on whether patients with PAH deficiency do not require treatment (when untreated Phe is below 360 μmol/L), or do require treatment (when untreated Phe is above 360 μmol/L), and on whether patients can be (partly) treated with the chaperone tetrahydrobiopterin (BH_4) (see Box 1) or can only be treated with diet as they do not respond to BH_4 (Keil et al., 2013). Note that the BH_4 deficiencies are distinct from the PAH deficiency and that they will not be examined in this thesis (but see Box 1 for definitions regarding these deficiencies). In this thesis, I refer to PAH deficiency when using the term PKU. It should be taken into account that some patients with high Phe levels, who do not have a genetically proven PAH deficiency or metabolically/enzymatically or genetically proven defect in BH_4 metabolism, might have mutations in the DNACJ12 gene (Anikster et al., 2017). Regarding PKU, it is beyond any discussion that when started early following neonatal screening, treatment with dietary Phe restriction prevents mental retardation (Blau et al., 2010). Therefore early initiation of treatment is vital.

Box 1. Definitions regarding PAH deficiency and BH_4 deficiencies based on Van Spronsen et al. (2017)

Tetrahydrobiopterin (BH_4) is a cofactor of phenylalanine hydroxylase enzyme (PAH) ,which converts Phe into Tyr, and also acts as a pharmaceutical chaperone that leads to a decrease of Phe concentrations and an increase of Phe tolerance, i.e. the amount of daily Phe intake patients can tolerate without an increase of blood Phe beyond the upper target Phe concentration.

The PAH deficiency is characterized by mutations in the gene encoding PAH. Some patients (20-50%) with PAH deficiency, especially those who have a high residual PAH activity, respond to BH_4 and are called BH_4 responsive. In these cases BH_4 serves as a chaperone protein. However, there are also patients who have BH_4 deficiencies. They have a defect in BH_4 synthesis or turnover and are treated mostly with BH_4 and neurotransmitter precursors.

Pathophysiology of PKU

Neurotransmitter shortages

Because the same amino acid carrier (L-amino acid transporter 1, LAT1) is used for transportation of Phe and other large neutral amino acids (LNAAs) across the blood-brain barrier, high blood Phe concentrations may result in high cerebral Phe concentrations and low levels of other LNAAs, even more so because the LAT1-system has a higher affinity for Phe than for the other LNAAs (Blau et al., 2010; de Groot et al., 2013; Pietz et al., 1999). Two of these other LNAAs, Tyr (of which there is already less available in blood due to the PAH deficiency) and tryptophan are the metabolic precursors of the cerebral neurotransmitters dopamine and norepinephrine, and serotonin respectively. Subsequently, decreased cerebral Tyr and tryptophan concentrations may lead to shortages of these neurotransmitters, while high cerebral Phe may also decrease the conversion of tyrosine and tryptophan in the neurotransmitters (van Vliet et al., 2016).

Dopamine is an important neurotransmitter for cognitive functions mediated by the prefrontal cortex (PFC). The dopamine innervation and receptor expression in brain are present early in development, continue to mature in adolescence and become more stable in adulthood (Money & Stanwood, 2013). The PFC is sensitive to minor changes / shortages of dopamine, because the dopaminergic neurons in the PFC have a faster firing rate and faster dopamine turnover than neurons in other brain areas. Minor loss of dopamine is therefore related to deficits in cognitive abilities (Christ, Huijbregts, de Sonneville, & White, 2010; Diamond, 1996). Serotonin is an important growth factor during embryogenesis and during life it is also important for cognitive processes, such as learning and memory, and mood (Sodhi & Sanders-Bush, 2004). A decrease of serotonin has also been associated with cognitive difficulties (especially those mediated by the orbitofrontal cortex), and with mood disorders and difficulties in social behaviour (Gellynck et al., 2013; Kiser, Steemers, Branchi, & Homberg, 2012; Kolb & Whishaw, 2003; Lesch & Waider, 2012).

White matter abnormalities

High Phe has a negative effect on the synthesis of myelin, e.g. due to inhibiting the myelin basic protein, and consequently on the function of white matter (Dyer, 1999; Fields, 2008). White matter is very important for brain functions, as it is involved in information processing, learning and communication between brain regions (Fields, 2008). Developmentally, the myelination process of white matter starts just after birth and continues at least into young adulthood. Brain areas that are myelinated earlier control the simple motor control or sensory functions, while the late-myelinating regions (the PFC being the latest) regulate the higher cognitive functions, leading

to refinement of behavioural and cognitive functions (Kolb & Whishaw, 2003; Nagy, Westerberg, & Klingberg, 2004). Furthermore, this myelination/white matter forms connections between neurons in different brain areas to ensure pathways for communication (Kolb & Whishaw, 2003). There are magnetic resonance imaging (MRI) studies describing white matter abnormalities in PKU (Cleary et al., 1994; Pearsen, Gean-Marton, Levy, & Davis, 1990; Pietz et al., 1996), which were found to be related to cognitive impairments in a subset of studies looking into such relationships (Anderson et al., 2007; Anderson & Leuzzi, 2010). White matter integrity has also been associated with Phe levels and executive functions separately, and some evidence even exists indicating that white matter integrity mediates associations between Phe levels and executive functions (Antenor-Dorsey et al., 2013; Hood, Rutlin, Shimony, Grange, & White, 2016; Mastrangelo et al., 2015). Furthermore, timing, adherence and consistency of treatment (keeping Phe under control and stable) affects white matter abnormalities, as measured with diffusion tensor imaging (DTI) (Hood et al., 2015; Peng, Peck, White, & Christ, 2014), indicating that proper maintenance of treatment is most important.

It is likely that due to these white matter abnormalities, atypical and reduced neural activity was observed in brain areas of patients with PKU, measured with functional magnetic resonance imaging (fMRI), which was related to poorer working memory, while functional connectivity was also decreased (Christ, Moffitt, & Peck, 2010; Christ, Moffitt, Peck, White, & Hilgard, 2012). The degree to which the abovementioned abnormalities in brain structure and function affect cognitive, behavioural and social outcomes in PKU is not yet fully known.

Treatment and metabolic control

The primary focus of treatment is blood Phe reduction. The basic treatment consists of a Phe-restricted diet. This implies a diet that restricts natural protein and necessitates supplementation of amino acids through a protein substitute with all amino acids except Phe. Other treatment possibilities to decrease the blood Phe concentration include tetrahydrobiopterin (BH_4), while pegvaliase (PEG-PAL) and gene therapy are still experimental and in various stages of development. Large neutral amino acid (LNAA) supplementation and glycomacropeptide (GMP) supplementation have a different position. Although an effect on blood Phe cannot be excluded, the idea behind LNAA supplementation is that the administered LNAAs compete with Phe at the level of the blood-brain barrier to block the entrance of Phe that uses the same transporter, resulting in a greater influx of the other LNAAs while the influx of Phe decreases. This way the high brain Phe concentrations can be reduced, while brain concentrations of other LNAAs can be increased or even normalized (Pietz et al., 1999; van Spronsen, de Groot, Hoeksma, Reijngoud, & van Rijn, 2010). LNAA

and GMP, however, are also believed to decrease the blood Phe level (Concolino et al., 2017; Matalon et al., 2007). LNAA may decrease Phe because a transporter more or less similar to the one active at the blood-brain barrier may also exist at the gut to blood barrier to some extent. GMP contains a large amount of specific LNAA and may therefore have the same effect. However, it is also believed to decrease the blood Phe concentration due to the fact that adherence to GMP would be better compared to existing protein substitutes, even when GMP contains some Phe after the manufacturing process of isolating GMP from other proteins (van Calcar & Ney, 2012). This means that it needs to be accounted for in a patient's daily Phe prescription. Enzyme replacement therapy with pegylated molecules of the enzyme phenylalanine ammonia lyase (PEG-PAL) for PAH helps catalysing the conversion of Phe so that the diet can be less strict (Longo et al., 2014). Phe levels have been shown to reduce (sometimes dramatically), but clear effects on cognitive, behavioural and social outcomes have not yet been observed.

At present there are two widely used forms of treatment for PKU: Phe-restricted diet and/or pharmacological treatment with BH_4 (see also Box 1) (Blau et al., 2010). These are also the only two forms of treatment used by patients participating in the study presented in this thesis. Apart from the fact that BH_4 is known as a co-substrate or cofactor necessary for the PAH enzyme to convert Phe into Tyr (as used in defects in BH_4 metabolism, which are explained briefly in Box 1 but which will not be further discussed in this thesis), BH_4 is also a chaperone that helps PAH to fold better (misfolding of the PAH protein leads to inactive or insufficient protein function) (Werner, Blau, & Thony, 2011). Only patients with at least some PAH residual activity (in approximately 20-50% of patients) can be aided by provision of pharmacological dosages of BH_4 as chaperone. This way BH_4 supplementation increases Phe tolerance, i.e. the amount of daily Phe intake patients can tolerate without an increase of blood Phe beyond the upper target Phe concentration (Blau et al., 2011). Generally, those who are BH_4 responsive, already have milder forms of PKU, caused by different types of mutations in the PAH gene (Keil et al., 2013). By means of genotyping of the PAH gene or a BH_4-loading test, it can be determined whether patients with PKU are BH_4 responsive (Anjema et al., 2013). In BH_4 responsive PAH deficient patients, it has been shown that administration of BH_4 was associated with improvements in quality of life, as adherence to the very strict diet becomes less important and therefore the impact of dietary restriction is lower (Bosch et al., 2015; Keil et al., 2013). It should be noted, however, that only some of the BH_4 responsive patients can drop the diet entirely.

Monitoring of metabolic control consists mainly of regular measurements of blood Phe concentrations. Children with PKU are monitored more often than adolescents and adults. In the first year of life children are monitored weekly, while

adults are monitored at least once a month, i.e. by sending dried bloodspot cards to the clinic. Adults with PKU visit their treatment centre once or twice a year, while children visit their centre usually every two-three months, depending on the clinic's or country's guidelines (Blau et al., 2010; Trefz et al., 2015; van Spronsen, Ahring, & Gizewska, 2009). The exact number of patients, especially adults, who are lost to follow up for instance due to inadequate transition to the adult clinic is unclear (van Spronsen et al., 2009; Vockley et al., 2014), although a survey among European and Asian healthcare professionals calculated that 15-19% of adult patients were lost to follow-up (Trefz et al., 2015) while another survey estimated a 48-77% loss of adults with PKU in the USA (Berry et al., 2013).

Treatment guidelines

The USA treatment guidelines were constructed by a non-Federal panel of experts during National Institutes of Health Consensus Development conferences (Camp et al., 2014; National Institutes of Health Consensus Development Panel, 2001). In Europe, the patients in the European Society for Phenylketonuria and Allied Disorders (ESPKU) wrote their first consensus paper in which they urged for the first European guidelines (Hagedorn, van Berkel, Hammerschmidt, Lhotakova, & Saludes, 2013). The first European treatment guidelines addressed five themes: 1) nutritional treatment and biochemical / nutritional follow-up, 2) adult and maternal PKU, 3) late diagnosed and untreated PKU, 4) diagnosis of PKU including treatment initiation and drugs, and 5) neurocognitive outcomes including psychosocial and neuroimaging outcomes and the role of treatment adherence (van Spronsen et al., 2017).

Treatment targets for Phe in children are relatively clear and similar across countries. A Phe concentration of 360 μmol/L is the most frequently used upper target level in childhood, i.e. for the first 12 years of life (van Spronsen et al., 2009). While in Germany an upper target level of 240 μmol/L is recommended in childhood (Burgard et al., 1999), the upper target Phe level in some European centres and countries is/was 500 μmol/L in children (van Spronsen et al., 2009). In adolescence and adulthood treatment targets between 600 and 1200 μmol/L have been advised (Blau et al., 2010). In the USA and Europe treatment for life is recommended. Whereas in the USA the most recent advice is to keep Phe below 360 μmol/L for all ages (Camp et al., 2014; Vockley et al., 2014), European guidelines advise to keep Phe below 360 μmol/L in the first 12 years and below 600 μmol/L thereafter (van Spronsen et al., 2017).

Although there appears to be relatively good consensus about upper therapeutic targets for Phe in PKU, it is important to note that both the USA and European recommendations were achieved without strong support from the literature,

especially if ages after childhood are concerned. Many studies had methodological shortcomings or examined specific and small subsets of patients. Furthermore, on many occasions, important information (e.g. about historical Phe levels) was missing. Moreover, inconsistent or mixed results were obtained, which may have been due to differences in study design or choice of instruments for neuropsychological testing. Thus, the main objective of this thesis was to look further into treatment targets for different ages, thereby using a comprehensive design covering outcome measures that have been used previously as well as new, potentially important outcome measures.

Executive functions, behaviour and social (cognitive) skills in PKU

Executive functioning, mental health and social (cognitive) functioning are the main outcome measures in this thesis. The most consistently impaired aspect of cognition in PKU studies is executive functioning (EF, i.e. a collection of regulatory functions underlying social-adaptive behaviour, to be further explained in the following paragraph). Mental health issues, particularly internalizing behaviour problems (e.g. anxiety and depression) are also reported in studies with PKU patients. Finally, as not only EF but also social cognitive abilities underlie social-adaptive behaviour (including psychopathology or mental health but also general social functioning), social cognition and skills were newly included as outcome measures of interest for this thesis (i.e. these have not been investigated before in PKU).

Executive functioning (EF)

EF consists of a number of higher-order cognitive abilities that are needed for goal-oriented and efficient behaviour. They regulate thoughts and behaviour in response to changing internal (i.e. cognitive) and external (environmental) demands. Examples of EF are inhibitory control, cognitive flexibility, planning and organizing. EFs start to develop in early childhood, i.e. basic working memory and inhibitory control skills, while the more complex functions, e.g. cognitive flexibility, strategic thinking and planning, develop in adolescence and continue to develop into young adulthood. The simple EFs are the building blocks of the more complex functions, they continue developing during childhood and become more sophisticated in adolescence and young adulthood. Working memory, inhibitory control and cognitive flexibility are often considered the core executive functions (Christ et al., 2010; Pennington & Ozonoff, 1996; Zelazo et al., 2003). Even when patients (particularly children) with PKU are treated well and tend to meet therapeutic targets, studies have shown that they exhibit difficulties with executive functioning compared to healthy controls (Albrecht, Garbade, & Burgard, 2009; DeRoche & Welsh, 2008; Huijbregts, de Sonneville, van Spronsen, Licht, & Sergeant, 2002; Moyle, Fox, Arthur, Bynevelt, &

Burnett, 2007). Associations between task performance (of tasks measuring inhibitory control, working memory and cognitive flexibility) and Phe concentrations have often been shown, but there is still a lack of clarity to which degree such associations are still evident beyond childhood (when Phe levels have been below the upper target levels throughout childhood) (Channon, Mockler, & Lee, 2005; Christ et al., 2010; DeRoche & Welsh, 2008; Huijbregts et al., 2002).

Mental health

Studies examining mental health in PKU have reported a higher incidence of internalizing behaviour problems compared to healthy individuals, without explicitly addressing whether these were clinically significant. Examples of internalizing behaviour problems are depression, anxiety, withdrawal and somatic problems. Studies have reported increased depression, anxiety, phobic reactions and poor self-image in PKU, especially in patients with high concurrent or lifetime Phe concentrations (Anjema et al., 2011; Arnold et al., 1998; Cappelletti et al., 2013; Smith & Knowles, 2000; Weglage et al., 2000). Externalizing behaviour problems are directed towards an individual's environment, like attention deficit/hyperactivity disorder (ADHD), aggression or conduct problems, causing impairment or interference in daily life functioning (Achenbach, Ivanova, Rescorla, Turner, & Althoff, 2016). Perhaps with the exception of ADHD, the inattentive subtype, externalizing problems have generally not been observed in PKU (Cappelletti et al., 2013; Smith & Knowles, 2000; Weglage et al., 2000). Neurotransmitter shortages in PKU might be related to mental health since dopamine shortage has been related to attention and EF problems (Christ et al., 2010), and serotonin shortage has been related to depression and mood problems (Brummelte, Mc Glanaghy, Bonnin, & Oberlander, 2016; Gellynck et al., 2013).

Social cognition and skills

Social cognition consists of mental processes necessary to perceive, decode, interpret and respond accordingly to social stimuli. Social cognitive skills underlie social interaction and social skills. Basic social cognitive skills are recognizing faces and emotions and one of the most important skills is the Theory of Mind (ToM). The ToM is the ability to understand what someone is thinking, feeling, knowing and what someone's intentions are considering circumstances and situations (Hughes & Leekam, 2004). Social skills are the abilities enabling interaction and communication with others in daily life, such as verbal and non-verbal communication (Gresham & Elliott, 1987). Social skills are connected tightly with executive control (Perner & Lang, 1999). Also, EF and social (cognitive) skills share some underlying neurobiology and neuro-anatomy (Arnsten & Rubia, 2012; Murphy, Smith, Cowen, Robbins, &

Sahakian, 2002). Certain mental health problems, such as depression and mood swings, are characterized by social deficits. Also, decreased serotonin levels have been associated with difficulties in social behaviour and social interaction (Kiser et al., 2012). As social cognition seemed such a central construct related to many observed problems in PKU, it was introduced in this thesis (and PKU research in general) as a potentially important outcome measure.

Main research questions

This General Introduction showed the importance of treatment and proper treatment guidelines for patients with PKU, to prevent pathophysiology and to ensure adequate EF, mental health and social functioning. Based on the abovementioned description of deficits in PKU, the existing literature and treatment guidelines for PKU, the following main research questions are the focus of this thesis:

1. *What should be the optimal treatment target for children with PKU (i.e. 0-12 years old) based on cognitive and behavioural outcomes? Is 240 µmol/L for children better than the currently most widely recommended upper target limit of 360 µmol/L?*
2. *What is the cognitive, behavioural and social profile of patients with PKU in different age periods?*
3. *Does the cognitive, behavioural and social profile of patients with PKU during adulthood relate to blood Phe levels of childhood and beyond?*

Outline of the thesis

In order to answer outstanding questions on therapeutic targets (at different ages) and to obtain a more complete picture of the behavioural phenotype associated with PKU, the Phenylketonuria-COBESO study (PKU-COBESO) was created and conducted in six Dutch university medical centres. It is a longitudinal (follow-up) and cross-sectional study focusing on COgnitive, BEhavioural and SOcial functioning (COBESO) of early and continuously treated PKU patients in relation to their (history of) metabolic control.

Chapter 2 will provide detailed information about the PKU-COBESO study. Preliminary results (obtained when data collection was still under way) concerning EF and mental health will also be reported in this chapter.

The main topic of **Chapter 3** is treatment targets and guidelines in childhood. Upper target Phe levels of 360 and 240 µmol/L in childhood will be compared, as these are the most frequently used recommendations. Executive functioning will be the primary outcome measure.

In **Chapter 4** the cognitive profile and mental health of adult PKU patients will be examined. The treatment target of 360 µmol/L will be used to create groups and to differentiate between patients with good and poor metabolic control. Part of this

chapter will address the clinical relevance of the results and the influence of BH_4 treatment on the outcome measures.

Longitudinal data will be presented in **Chapter 5** where the relationship between lifetime metabolic control and executive functioning and executive motor control assessed at two points in life (childhood and adulthood) will be investigated. Mental health in adulthood will be examined as well.

Results on social cognitive functioning and social skills of PKU patients will be presented in **Chapter 6**. A distinction will be made between children, adolescents and adults with PKU, and they will be compared to healthy matched individuals. Associations with metabolic control will also be investigated.

Chapter 7 will give insight into the question whether the Behavior Rating Inventory of Executive Functions – Adult version (BRIEF-A) is a useful instrument in day to day care of adult PKU patients, as there is a need for easy assessment of EF in daily life. Results from the BRIEF-A will be compared to results from the Amsterdam Neuropsychological Tasks (ANT), a computerized test battery used in a lab setting.

The General Discussion will address the three main research questions posed in the General Introduction, while future directions will also be provided in **Chapter 8**. Finally, **Chapter 9** will provide a summary of this thesis.

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